Research Article

A dual frame survey to assess time– and space–related changes of the colonizing wolf population in France

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Abstract

The wolf recovery in France dates back to 1992, following the natural range expansion of the remaining Italian population since the late 1960's. Facing a high level of interactions between wolves and sheep breeding, decision makers had to quickly balance the need for managing livestock depredations with the conservation of wolves as a protected species. The French authorities therefore required a reliable assessment of changes in the species range and population numbers, as well as a reliable monitoring of depredations on livestock, all being key variables to be further included within the governmental decision making process. Because of their elusive behaviour, high mobility, and territoriality, applying a standard random sampling design to the monitoring of a wolf population would lead to almost no chance of collecting any signs of presence. In order to increase detectability, we use a dual frame survey based on two spatial scales ("population range" and "reproductive unit") investigated sequentially thanks to a network of specifically-trained wolf experts distributed over 80000 km^2 to collect the data. First, an extensive sign survey at a large scale provides so-called cross-sectional data (pool of signs from unknown individuals for a given year), thereby allowing the detection of new wolf occurrences, new pack formations, and the documentation of geographical trends. Secondly, an intensive sign survey within each detected wolf territory, based on standard snow tracking and wolf howling playback sessions, provides some yearly updatable proxies of the demographic pattern. The combination with a non invasive molecular tracking provides longitudinal data to develop mark-recapture models and estimate vital rates, population size and growth rate, while accounting for detection probabilities. The latter are used in turn to control for proxies' reliability and to implement demographic models with local population parameters. Finally, wolf activity patterns in connection with predator-prey dynamics is investigated through a pilot study carried out with both wolves and four ungulate preys radio-collared. A particular attention is paid to checking the reliability of presence sign data, as well as improving the cost-efficiency ratio of the monitoring. Finally, these results are also used by the government as one of the components in the decision making process related to the management of coexistence with wolves.

Introduction

Wolves have recovered in the French Alps since the early 1990's, as a consequence of the range expansion of the Italian population (Ciucci et al., 2009; Lucchini et al., 2004). Non-invasive genetics have suggested a unidirectional gene flow from the Apennines to the Alps with a small number of founders, explaining the actual genetic diversity of the alpine population (Fabbri et al., 2007). Wolf recovery in France takes place in an area of intensive agricultural activity with large flocks of sheep grazing in summer pastures. This resulted in strong interactions between wolf presence and sheep breeding activities. The first aim of the French authorities was to get reliable estimates of wolf distribution, population trends, and depredations levels, in a way to base the decision making process on yearly updates of the species' conservation status. The national wolf action plan (MEDEEM, MAP, 2008) aims at fulfilling the requirements of European Directives relating to wolf conservation (Council of Europe, 1992), while still allowing sheep herding to be economically and socially viable.

Following Pollock et al. (2002) recommendations, the choice of the monitoring system is designed to provide (1) robust estimates of population dynamics (e.g. abundance, population range trends, survival rates, etc.), and (2) yearly updates on the conservation status of this protected species, as a basis for management. The monitoring system also has to enable quick detections and reports of dispersing vagrants in newly colonized areas so that decision makers could politically anticipate social reactions and implement management actions (e.g. preventive measures to decrease attack rates on livestock).

Monitoring large carnivores is notoriously difficult because they are elusive, occur in low densities, although they are clustered in space due to their territorial behaviour (e.g. packs), and are able to disperse over hundreds of kilometres within few weeks (Boyd and Pletscher, 1999). Finding a wolf, or simply a sign of its occurrence

over its territory means dealing with rare statistical events. Radio-telemetry is widely used over the world for monitoring small populations of wolves or (on specific study sites) for estimating territory size and pack sizes (Jedrzejewski et al., 2001), documenting dispersal patterns (Kojola et al., 2006), breeding status (Ausband et al., 2010) or kill rates (Sand et al., 2005). But such invasive techniques are usually inappropriate to monitor a population as a whole (Kunkel et al., 2005) due to labour intensive and expensive methods that cannot cover a sufficient pool of marked animals over a large scale. That's why non-invasive monitoring (i.e. not requiring the animal handling) are usually preferred since they perform better at the population level with elusive species (Linnell et al, 1998; Long et al., 2008).

The ability to detect geographic or demographic patterns is a function of both the extent and the grain of sampling investigations (Wiens, 1989). Therefore the wolf monitoring requires one to work at different spatial scales. A large spatial scale approach is needed to target the whole population range, including both territorial individuals and dispersers, so as to reliably assess demographic and geographic population trends. A smaller spatial scale is however also needed, to specifically investigate demographic dynamics within the reproductive units (packs) and, from this subset of data, to derive proxies of demographic population trends in a cost effective way regarding field effort.

We propose a dual frame survey based on a sequential data collection (*sensu* Christman 2004) involving first an extensive survey at large ("population" level) scale and then an intensive survey at a small ("reproductive unit" level) scale once a decision rule is booked at the first level. A spatially-controlled network of field people trained to detect signs of presence allows us to compile two sources of data – i.e. cross-sectional vs. longitudinal – both collected at each spatial scale. The present paper describes how the monitoring scheme is implemented, what kind of data are collected and how those data are used to assess wolf conservation status in the French Alps and to feed the decision-making process to outline management actions.

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Materials and Methods

Study area

The study area (Fig. 3a) covers all the French part of alpine and pre-alpine formations (from 4° 44'E, 43° 05'N to 7° 05'E, 46° 20'N). Mountainous areas are mainly concerned, but lowlands are also included. Habitat (including coniferous forest, shrubs and pastures) is influenced by Mediterranean climate in the South, while habitat in the Northern part of the study area is continental with mixed forests, alpine pastures and rocky mountains.

Chamois (*Rupicapra rupicapra*), mouflon (*Ovis ammon*), roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*), ibex (*Capra ibex*), wild boar (*Sus scrofa*) and domestic sheep (*Ovis aries*), are the main species constituting the prey community. Sheep husbandry is a major agricultural activity with about 900000 sheep bred for meat production (Ernoult et al., 2001). The practice is based on an extensive use of natural mountain pastures during the summer period (transhumance). Outdoor sheep breeding may occur all over the year in southern regions of the study area, while it is restricted to a 3-4 months (summer) period in the northern part of the Alps.

Monitoring survey design

The wolf monitoring in France is carried out since the first confirmation of the species' presence in 1992 (Houard and Lequette, 1993; Valière et al., 2003). The monitoring aims at investigating the population dynamics of the species to assess its conservation status.
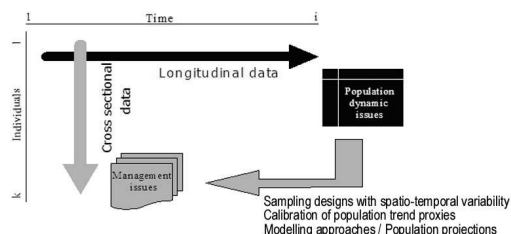

Figure 1 – Investigation of two different sources of data in order to reach both management requirements and robust population dynamics results to be used in the decision making process.

As estimates of population dynamic parameters usually require long term monitoring, it often does not fit with the management issue of such a controversial species that need yearly updates of the population status. We therefore combine different sources of data (Fig. 1) to achieve both management and population

dynamic issues: 1) cross-sectional data – i.e., referring to the sampling of a pool of unknown individuals at a given point of time – allow estimating changes from one year to another in the species' distribution range or new pack formations, and (2) longitudinal data – i.e., repeated observations of known individuals over time – are needed to control for variations in the detection probability, to estimate demographic growth rates and survival rates, that can further feed demographic models. The results of demographic models can be in turn implemented into the management issues, for example when calibrating proxies derived from the cross sectional datasets.

The collection of cross sectional as well as longitudinal datasets is based on a network of field observers specifically trained during a 3-day teaching course, covering species identification and sign survey methods. We paid a particular attention to the pooling of different socio-professional backgrounds (ecology, environment, agriculture, hunting) of the experts. This improved the social acceptance of the results, while improving the chances of detecting any wolf occurrence since members have different patterns of field activities. These field experts (n = 1024 in 2009) come from governmental or non governmental organisations (game and wildlife services, parks, hunting associations, forestry offices, naturalists, farmers, etc.) the core technical group being composed by 67% of "wildlife related" professionals from governmental agencies, who are available anytime it is needed. Among NGO's, 10% are professionals, the remaining 23% are people who do not claim for any belonging to wildlife related GO or NGO such as hunters, naturalists, mountain guides or shepherds (Fig. 2).
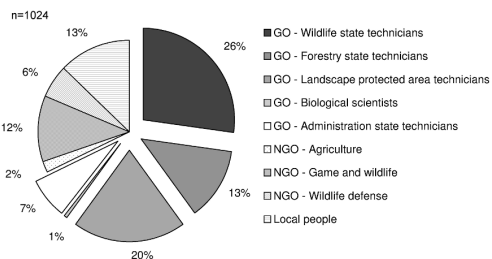

Figure 2 – Socio-professional background of the experts belonging to the Wolf/Lynx Network (N= 1024 people in 2009).

To optimize the cost/benefit ratio of the survey effort, the network of field experts is implemented sequentially over space, based on the sequence of documented wolf signs. First a baseline monitoring is carried out in all wolf free districts, based on state

Table 1 – Wolf population dynamic issues investigated through a scale dependant monitoring design of the wolf population combined with two data frame collections.

	Cross-sectional data frame	**Longitudinal data frame**
"Population range" scale	Detecting new area of presence	Dispersal pattern
	Detecting pack formation	Genetic structure
		Survival rates and population size
"Reproductive unit" scale	Proxies of demographic population trend (minimum wintering pack size, reproduction occurrence…)	Home ranges and activity patterns
		Predation pressure
		Pack social structure

game-wardens from the National Game and Wildlife Agency (ONCFS) already dispatched all over France for environmental policy and wildlife monitoring (n = 10-18 per administrative county of average size S = 5718 km^2, SE = 198). Despite such a weak sampling pressure, they can detect new occurrences since they are regularly distributed in space for their daily outdoor wildlife activities (monitoring or policy). Once a vagrant wolf is regularly detected at a given place (i.e., it is no longer dispersing), training sessions (n = 30-70 volunteers) are organized to increase the survey effort in order to apply the following dual frame monitoring survey. Each year, some more volunteers are trained, so that they regularly increase in numbers. The network activity is coordinated and promoted regionally by a wolf referee in charge of controlling the technical reliability of each reported sign of presence, according to a standardized approach (see "sign survey and molecular tracking"). All the collected data are pooled within a single database, managed by our ONCFS research team.

Evaluating the conservation status of a species requires the estimation of a large panel of demographic parameters such as gene flows, trend of the number of reproductive units or population range, estimation of home ranges of each pack, etc. However, all these parameters may require investigation at different spatial scales. Because of logistical constraints, one may be bound to stratify the sampling design at a limited number of different spatial scales to fit with the biology of the species a minima. We defined two spatial scales as i) the "population range" scale that aims at recording new wolf occurrences and monitor distribution range through time and ii) the "reproductive unit" scale that aims to investigate population dynamics estimates (Tab. 1).

The dual frame monitoring survey is based on this two spatial scales, investigated one after the other (Tab. 1). First, an extensive and opportunistic sign survey is conducted all the year round by the field experts over the whole population range (hereafter called "population scale"). The sampling effort is not quantitatively controlled but is spatially organized,

focusing on the distribution of the experts over the area to be surveyed in order to have as few zones as possible without any expert. This extensive survey aims at detecting new wolf occurrences, whether documented by a hard fact (photograph, genetic analysis) or by other signs of presence (visual observation, depredation on livestock, wild prey, prints, etc.). To check the extent of sampled area according to the spatial distribution of experts, we analysed the frequency distribution of Euclidean distances between each geo-referenced sign of presence and the related position of the expert's home place who collected it. The mean distance and associated 95% confidence limits are calculated and the upper boundary subsequently used as a buffer around each expert home place to map the most likely coverage of the network. The map aims at depicting whether the distribution of the experts over space is relevant to monitor the geographical distribution of the species over most of the population range (i.e., the alpine area) or not. We estimated the trend of geographic distribution (EDR) using the projection of each sign of presence accounting for different grid size and shape. Because the choice of cell area types and units may influence the pattern of distribution range (Marboutin et al., 2010), the yearly change of the estimated distribution range (EDR) is evaluated as the mean deviations for each year from the mean EDR over all years and 4 cell types (buffer and grid shape of 6, 25, 50 and 100 km^2) of cell area units.

At that step, the specific cost devoted to the detection of wolf presence can hardly be quantified because of the opportunistic nature of the survey. Apart from the systematic control of each depredation *in situ* that requires on average 59 (SD = 21) day×man×wolf territory^{-1}×year^{-1}, all the other signs of presence are recorded "for free" during the wildlife policy investigations as a whole or during other outdoor activities (hunting, mountain skiing…).

As soon as wolf signs have been recorded over two consecutive winters at a given site (with at least one genetic identification), then it is stated as an area with sedentary presence of the species – i.e., a territory held by a single wolf, a pair or a pack. The
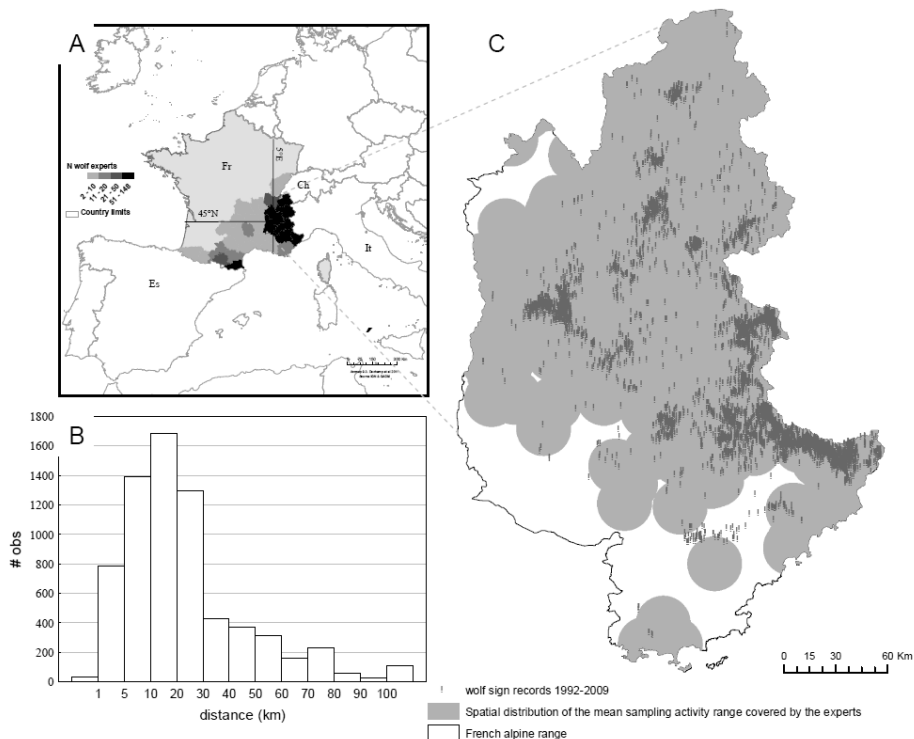
Figure 3 – A) Distribution of the field experts belonging to the wolf/lynx network by districts in France and localization of the study area used for the test (French alpine range); B) Distribution of distances at which signs are recorded relatively to the expert home place ; C) Mean spatial coverage of the sampling effort displayed by the wolf-expert network in the alps when looking for wolf signs of presence (excluded damages on livestock that are exhaustively controlled in the field once appeared anywhere). The buffer radius has been computed using the mean (log$_e$ transformed) value of the distributions in box B.

presence detected over two consecutive winters is the decision rule to switch to the second step of the monitoring design. An intensive survey is then carried out within each of these wolf territories (hereafter called "reproductive unit scale") once identified by the first step. The sampling effort is then spatially and quantitatively controlled, with explicit sampling scheme for snow tracking (transect sampling) during winter and wolf howling (point sampling) during summer. The winter survey (from November to March) aims at recording minimum pack sizes, i.e., the highest minimum count of pack members detected while moving together, and pack composition (based on non invasive genetics using faeces and urines collected along the transects). Snowtracking sessions are performed 48 hours after each snowfall by ski or snowshoe-riding, using regularly distributed transects so as to sample the whole expected wolf territory and to optimise the track detection (Beier and Cunningham, 1996; Linnell et al., 2007), while still ensuring people's safety from avalanche risks. The transect

density ranges from 1/30 km^2 to 1/50 km^2, and mean transect length is about 12 km. Transects are repeated at least 2 up to 6 times per winter, the numbers of replications varying between sites according to the local recurrence of favourable conditions for sign detection. Once a wolf track is encountered, the transect is left if wolves don't follow it, and we follow their tracks as long as possible to favour the records of possible additional information (group size, scat collection or prey remain). Doing that way, we are not able to compute the total number of km sampled (since not all the people involved are using GPS "tracker"). The distribution of the minimum number of different animals along each track gives the estimation of the minimum pack size. We use the sum of each maximum recorded pack size (i.e. minimum numbers detected) as a proxy of the demographic trend of the population, knowing that the annual rate of increase $\lambda = N_{t+1}/N_t$ derived from this proxy matched well with that estimated from mark-recapture models (Marboutin and Duchamp, 2005).

The summer survey (from August to September) aims at documenting breeding events. Wolf howling playback sessions (Harrington and Mech, 1982) are conducted on a series of sampling points scattered over each pack territory previously detected by the first survey level. We started wolf howling survey in 2003. The sampling effort averaged 12.9 (SD = 10.8) points × session^{-1} × pack^{-1} totalising from 76 points in 2003 up to 411 points in 2009 all over the French alpine range. The observed variability in the sampling effort between packs mostly depends on the number of replications needed to be successful with a maximum of 6 replications among each pack. We applied the wolf howling sampling from August the 1st to September 15th. The choice of the period corresponds to the time i) when pup vocalizations can be distinguished from adult ones (Harrington, 1986; Nowak et al., 2007; Sebe et al., 2006) and ii) when pups stay at rendez-vous sites before moving with other members of the pack (Gazzola et al., 2002; Packard, 2003). We use the proportion of packs that reproduced among the detected ones to identify the trend of the reproductive pattern of the population assuming constancy over years in the difference between response rates of a pack with vs. without pups.

Both non invasive molecular tracking and indirect sign survey are however not accurate enough to investigate wolf activity patterns or document home ranges of packs, mainly owing to the space-dependent sampling effort devoted to faeces collections. Using new GPS/GSM devices is conversely very relevant to understanding e.g. spatial activity patterns or the role of predation in shaping the prey community (Merrill et al., 2010). Studying the interactions between predators and preys in France involves working with many different prey species at a time (roe deer, red deer, chamois, mouflons), elsewhere subjected to various sources of demographic variations (climate, density, diseases, hunting, and predation). One pack located in the Mercantour mountains (6° 54'E, 44° 6'N) is used to study the wolf impact in a multi-prey sytem. The four mentioned ungulate species are intensively marked since 2004 with mortality sensor VHF collars (Followitt Inc). Mortality signals from all animals are checked 3 times a week. In case of mortality, the carcass is controlled in the field to determine whether it was predated or not, based on characteristics of neck bites and of consumption. The data are subsequently used for capture-recapture modelling. Beside the prey monitoring, we captured 3 females wolves using leg hold traps (Beslile™) or helicopter darting and fitted with GPS/GSM collars (Wildcell SG - Loteck Inc). Immobilization protocol followed Arnemo and Fahlman (2007). GPS data were scheduled every 6 hours all along the year associated with an intensive 30-min locations from 6:00 PM to 5:30 AM (GMT) during 13 days per month to monitor prey selection (Manly et al., 2002), kill rates (Zimmerman et al., 2007) and estimate the spatial pattern of the predation pressure with GPS data.

We used the fixed kernel method to estimate the 95% home range (Lichti and Swihart, 2011; Worton, 1989) with a smoothing h factor estimated for each collars with the least squares cross-validation method (Horne and Garton, 2006).

Sign survey and molecular tracking

Any detected wolf sign of presence is collected, including prints, visual contacts, prey remains, hairs, scat or urine deposits, howls or dead wolves. We emphasize the need for using all possible categories of signs of presence, provided that all are controlled and validated using standardized criteria to ensure technical reliability. The same technical forms (one for each category of signs) are used by each of the wolf experts, to describe what has been recorded based on several technical criteria (e.g colour, body size, behaviour, tail length for a visual contact, or pad size, length and alignment along a wolf track, or bites and consumption characteristics for prey remains). Each sign is then submitted to a standardized validation process using the following rule: if less than half of the technical criteria required are fulfilled, the sign is classified as "undetermined"; if more than half of the criteria are fulfilled, all relating to wolf characteristics, the presence sign is classified as "reliable"; if one of them mismatched with a wolf characteristic, the sign is classified as "unreliable" and discarded. Damages to livestock are considered as signs of wolf presence. All depredation cases are controlled in the field by a wolf/lynx expert of the network following fifteen criteria (as for wild preys) related to bites, consumption characteristics and surrounding events (Dahier and Lequette, 1997; Francis, 2004). The same rule is applied as for other signs of presence for the validation process. To summarize, only those signs of presence with enough technical criteria and without any discrepancy from wolf characteristics are taken into account for further analysis.

Monitoring a set of marked individuals over time is the key point to account for detection probabilities. Instead of catching wolves, the non invasive genetic analysis of scats, urines, hairs, tissues from dead animals, blood collected opportunistically, along wolf tracks and at rendez-vous sites allows to get larger numbers of "marked" animals than in, e.g. classical telemetry-based studies (Waits and Peatkau, 2005).

All scat, hair, tissue or urine samples are submitted to genetic analysis (Taberlet et al., 1999; Valière and Taberlet, 2000). A set of 7 microsatellites (including sex) FH2054, FH2096, FH2137, FH2140, FH2161, PEZ17, YChr, have been selected to perform individual discriminations with an observed probability of identity P_{id} obs < 0.001 (Valière, 2002). These microsatellites are used in common between French, Swiss and Italian labs to allow for transboundary genetic monitoring of this alpine population unit (Fabbri et al., 2007; Linnell et al., 2008). The multitube approach is used with 8 independent PCR replicates per locus. A quality index is assorted to each sample based on the stability of allelic frequencies at all loci over the 8 replicates to prevent misidentifications (Miquel et al., 2006). The molecular tracking makes use of all the samples collected, whether by the extensive or intensive surveys.

These individuals are genetically tracked over years, and fits well with mark-recapture (MR) modelling for estimating demographic parameters. We use multievent MR models on seasonal recapture occasions (4 month periods) to estimate survival rates and detection probabilities while accounting for time effects on both parameters and detection heterogeneity among individuals (see Cubaynes et al. 2010 for details).

Results and discussion

Monitoring survey design with the Wolf Network

The wolf expert network is implemented over the large south eastern part of France (Fig. 3a) with 8 up to 140 experts per administrative units depending on the wolf detection step (wolf free area vs already known wolf pack presence). Overall, 14849 wolf signs of presence have been validated during the 1992-2009 period. Almost half (47%) of the field experts did not find any wolf sign for a given year, partly because several of them did not prospect inside of already known wolf territories (passive survey). Based only on those who reported at least one presence sign, the mean yearly reporting rate was 1.4 (95% CI = 0.3-7.0) wolf signs×year^{-1}×expert^{-1} up to 5.1 (95% CI = 4.0-6.4) wolf signs×year^{-1}× expert^{-1} (best year), with a maximum of 135 signs recorded by the same expert for a given year. Within the alpine sub-dataset, the distribution frequency of distances between the sign position and the administrative location of the expert who found it, was highly skewed (Fig. 3b) enlightening that most of the experts are short range sampler while few of them are long range field observers. Once $\log_2$ transformed, the average expert activity range was 15.8 km (95% CI = 15.33-16.11) around his administrative location (working or home place). Using the upper 95% confidence limit as a buffer radius to all corresponding expert locations, 81.5% of the French alpine range is covered (Fig. 3c) by network of experts representing a surveyed area of 42073 km^2.

One can reasonably assume that nearly everywhere in the Alps, the probability of detecting a wolf presence sign is non-null, given the species' presence. There are some uncovered gaps, possibly surveyed by "long range" experts, but it is always difficult to differentiate between true absence and undetected presence of the species. The obtained results do not account for possible variations of detection probabilities between years and therefore should be considered only as a proxy of the wolf distribution trend assuming no variations between years. These points at the crucial need for a well spatially designed and controlled network with trained people working within the same framework in order to minimize detection variability, and detect the wolf presence as fast as possible, especially if management issues are concerned.

Cross-sectional data at "population" and "reproductive unit" scales

At the population scale, all recorded sign types do not equally contribute to the presence area estimate (Tab. 2). The damages to livestock represent almost half of the sign records, and contribute the most to identify newly colonized areas. These areas are usually confirmed by additional hard facts (DNA based faeces, hairs, tissues or blood samples) on average 1.5 years later on. Visual contacts make up only 10% of the dataset, but also frequently contribute to the first documentation of the species' occurrence in a new area. Hard facts and tracks represent 33% of the total validated dataset, and are reported mainly from already known wolf territories. If only hard facts such as genotyping or photo-

Table 2 – Proportion and validation rate of the different types of sign recorded to detect the wolf presence and their relative contribution in the detection of newly colonized areas from 1992 to 2009.

	% validated among each sign type	N validated	Contribution to the first detection of a new area of presence (%)	% occurrence / total validated
Track	70.9	2417	8.9	16.2
Visual contact	62.0	1511	26.4	10.1
Wild prey remains	51.5	1082	7.6	7.3
Faeces, urine or blood[1]	37.4	2461	5.6	16.5
Howls	61.1	276	0.5	1.9
Hairs[1]	6.1	16	0.2	0.1
Canis skull or tissues[1]	56.6	43	1.7	0.3
Livestock damage	73.3	7043	49.1	47.3
Total	-	14849	100	100

[1] mtDNA-based identification only.

graphs had been taken into account, only 10% of the yearly distribution range would have been documented. This pattern of data distribution over space and time points to the need for taking into account all validated data sources (whether damage to livestock, documented visual contacts or genetic analysis) in order to provide a reliable view of the wolf distribution. Standardizing the evaluation of technical reliability for each data record is a powerful way to discard false positives (*sensu* Rondinini et al. 2006) and yields a conservative estimate of the population range. For example, almost 30% of the depredation cases, 38% of the visual contacts and 49% of the prey carcasses were discarded because they were either insufficiently documented or inconsistent with the species identification. To prevent possible remaining false positives, we also used a conservative decision-rule to assess the presence of a sedentary group, which had to be documented during two consecutive winters within the same area, together combined with at least one genetic identification. A balance between too restrictive rules and too permissive interpretations is of particular importance, to make all stakeholders confident with the scientific approach, and to avoid over-interpretation of the data.

Based on differences between the yearly estimate of population range and its grand mean when averaged over the period 1996-2006 (Marboutin et al., 2010), the estimated distribution range increases by ca. 10 times in 10 years (Fig. 4a) but non linearly over years. The yearly rate of increase of the distribution area ranges from $\lambda_{edr} = 2.00$ at the beginning of the colonisation in 1997 down to $\lambda_{edr} = 0.98$ in 2006. The slight saturation phase since 2004 could suggest i) a continuous decrease of available space in the Alps tending to increase interactions between packs and therefore slowing down the speed of new pack formations in between already settled ones, and ii) large natural or anthropogenic barriers surrounding the alpine range (despite wolves can surrogate that constraint), both slowing down the speed of the colonization process.

At the "reproductive unit' scale, the intensive monitoring is carried out in each previously detected wolf territory, once the rule about presence during two consecutive winters is fulfilled. From the total transects' length planned (60 km over one pack territory in 1994; 1300 km over 27 territories in 2010), the mean length of wolf tracks followed per territory and per winter ranges from 4.1 (95% CI = 2.6-6.5) km to 9.1 (95% CI = 6.9-12.2) km depending whether the pack is transboundary or not ($F_{(1, 163)} = 8.51$, $p < 0.01$). The number of detected signs of presence depends on whether the territory is held by a breeding pair or not ($F_{(1, 90)} = 23.71$, $p < 0.001$). The mean wintering minimum pack size detected with snowtracking is 3.8 (SE = 0.11) wolves $\times$ pack^{-1} $\times$ winter^{-1}. The pack size do not vary between winter ($F_{(1, 18)} = 1.23$, $p = 0.24$).

During summer, the pack detection rate (p_{pack}) averages $p_{pack} = 0.67$ when using wolf howling,

among which pups are detected in 68% of cases (Fig. 4c). The proportion of packs detected with pups can be regarded as a sound index of breeding success, assuming the difference between the probability of detecting breeding packs and that of detecting non-breeding ones is constant over time. Although the number of pups alive after the first winter would be a better estimator of the actual recruitment rate of the population, this high and more or less stable proportion of detected packs that bred corresponds to a usual reproduction pattern of colonizing populations. As any field protocol, the wolf howling survey is quite time consuming but its cost to benefit ratio may however be improved when using it also to locate rendez-vous sites and subsequently collect the faeces of pups. This, in turn, strongly improves age-related survival analysis, or documenting dispersal patterns of the yearlings.

Longitudinal data at "population" and "reproductive unit" scales

At the population scale, 4408 samples have been submitted to genetic identification since 1993. Mt-DNA sequencing assigned 65% of the samples to the *Canis lupus* species (others being either unsuccessful PCR amplifications or other species), among which 64% were successfully genotyped (Fig. 5). The rate of unsuccessful PCR amplifications (15.2%) points to the low quality and quantity of DNA extracted from non invasive samples (scat, urine, hair) that are submitted to environmental deterioration before collecting them. Most of the non targeted species comes from samples collected in potential new areas of presence. The experts typically collect scat samples regarding their size, shape and visible macro-element constituting them (prey hairs, bones...). But we asked experts outside of already know wolf area for collecting them even if the faeces morphology is not a wolf typically scat in a way to maximise the detection of the species.

From 1992 to 2009, 292 reliable genotypes have been identified and tracked over years. Based on this genetic monitoring, the best multi-event Capture-Recapture model estimates the survival rates (all age classes included) at 0.75 (95% CI = 0.54-0.94) up to 0.90 (95% CI = 0.71-0.98)
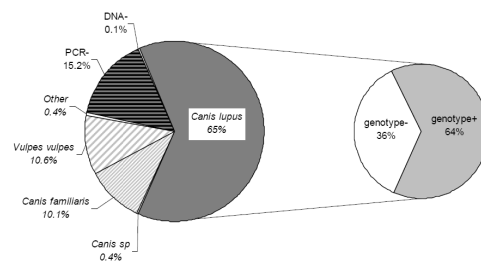

Figure 5 – Proportion of successful non-invasive genetic analysis in determining the species (mtDNA reverse sequencing) and subsequent reliable genotype (microsatellite DNA with global quality index > 0.6) based on samples of faeces, hairs, blood or tissues (N = 4408 samples submitted to genetic analysis). "PCR-" are unsuccessful Polymerase Chain Reaction amplifications, "DNA-" are samples with successful PCR amplification but unreadable DNA sequences.

depending on whether individuals belong to a weak vs high detectability class (Cubaynes et al., 2010). A strong heterogeneity in recapture rate between individuals was indeed noticed, with few of them being regularly detected over years (with a probability of detection ranging from 0.64 to 0.86 according to the season), and others being detected only once or twice (probability of detection ranging from 0.10 to 0.22 according to the season). Accounting for that heterogeneity in recapture rates, the population size (N = 126, 95% CI = 85-280 in 2003) is then re-evaluated by 27% compared to standard Cormack-Jolly-Seber models without heterogeneity (Fig. 4b). The power of assignment tests to infer about kinship was unreliable with that set of 7 microsatellites, so that we were not able to estimate age related survival rates. The sources of this heterogeneity in the detection probability still have to be identified, in order to separate the biological components (territorial vs. vagrant, social rank, age class) from the methodological ones, due to the very nature of a non-invasive survey of an elusive species (genotyping errors despite replications, non homogenous sampling design...) over such a large area and long time frame. Modelling directly the population rate of increase instead of population size seems more appropriate to assess the wolf population status because of its robustness over the variations of detection heterogeneity among individuals. This additionally yields to demographic models with less number of parameters
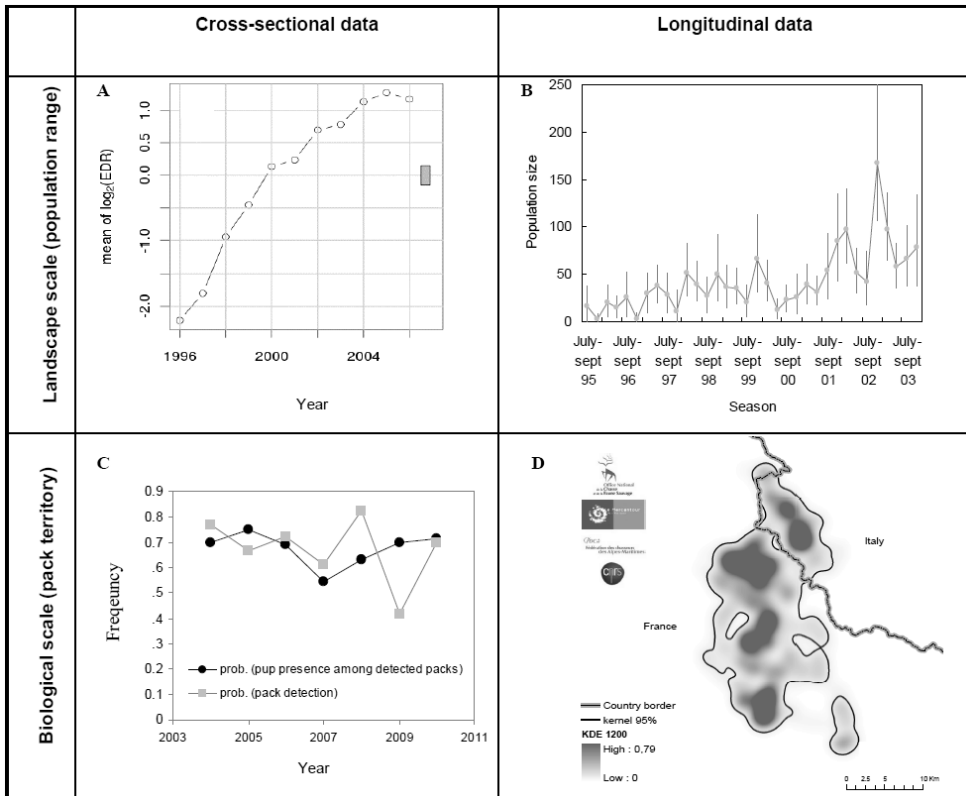
	Cross-sectional data	Longitudinal data
Landscape scale (population range)	A	B
Biological scale (pack territory)	C	D

Figure 4 – Examples of results obtained with the dual frame survey designed for the wolf monitoring in France in conservation biology, or population dynamics depending on the spatial scale and data type collection. A) trend in the estimated distribution range (EDR) of the wolf in France based on the model expressed as the mean deviation of EDRt combining different grid size and shape – see Marboutin et al. 2010; B) Estimated wolf population size since 1995 based on mark-recapture data using non invasive genetic accounting for detection heterogeneity – derived from Cubayne et al. 2010; C) Temporal trend in detected reproductive packs using wolf howling sampling - Duchamp et al. unpublished); D) Home range (95% kernel estimate) of a GPS collared female wolf monitored within the "Haute Tinée" pack (44° 15' 27.80N – 6° 55' 14.20E) to estimate the pattern of kill rates and impact of wolves on the prey community (French predator-prey project, Unpublished data).

and subsequent higher precision of the estimations (Marescot et al., 2011).

At the "reproductive unit" scale, the wolf howling sessions provide locations of homesites on which pup scats can be easily collected for following genotyping. This early detection of the pup genotypes gives the opportunity to monitor dispersal patterns for understanding the colonization process. Thanks to the GPS-based tracking of three females belonging to the same pack (caught in 2009, 2010 and 2011), fine-grained behavioural data could be gathered. Each of them was followed for 6 months since July, March and April respectively. The first was a non breeding female. The second and third ones were the alpha females of the pack (respectively in

2010 and 2011). The home range (95% kernel) of the first non-breeding female was 352 km² (Fig. 4d), whereas that of the second and third breeding females was 156 km². The alpha status of those two females that bred during the monitoring explains the reduced summer home range. The point density associated to the kernel estimates provides a spatial measure of the wolf predation pressure that could be further included in wolf-predation models (Fig. 4d). The set of 330 ungulates VHF-tagged within the pack area, compared to more than 900 ungulates marked and monitored within another wolf-free area will allow comparing survival rates with or without predation, once accounting for density dependence and environ-

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mental stochasticity. The ultimate goals of this study are i) to estimate whether the prey mortality due to predation is additive or compensatory to other sources of mortality, and ii) to evidence the indirect effects of predation on space use and demographic performance of preys.

Targeting the whole alpine population range

Fabbri et al. (2007) demonstrated a mostly unidirectional gene flow from the Apennine region in Italy to the Alps during the early phase of colonization. Twenty years later, 33 packs have been documented both in the Italian and French Alps (Wolf Alpine Group, 2011). They for sure contribute much more now to the demographic performance and genetic exchanges than the contribution of dispersers from the Italian Appenines. Since several packs have transboundary territories and several dispersers have been documented between France, Italy and Switzerland (Marucco, 2010), the "alpine' wolf population can be defined as a demographically speaking functional unit (Linnell et al., 2008). A Wolf Alpine Group (WAG) has been created in 2001, gathering the biologists and geneticists of the three countries in order to target this functional unit as a whole. Germany and Austria teams joined recently as some dispersing individuals have been detected there (Rauer, *unpublished data*). So far, the group has contributed to the harmonization of genetic approaches, the edition of periodic maps of pack distribution to attention of the European commission, and the field standardization of data collection (Wolf Alpine Group, 2004, 2008).

Reliability of a large scale monitoring of elusive species

The choice of the monitoring design is always a key point when addressing conservation biology issues, especially for elusive species that live at low densities, express territorial behaviours and are highly mobile. It is also crucial when mapping distribution range or estimating demographic trends, that the selected sampling scheme can target the whole functional unit of the population. The challenge of any wolf monitoring is to get a reliable sampling design able to cope with rare statistical events, such as signs of presence, at the population scale. These data must fit the requirements both for population status assessment and for local management of conflicts, particularly regarding new area of colonization. Recent methods have been developed to model occupancy rates or counts from data with imperfect detection or zero-inflated data sets (Cunningham and Lindenmayer, 2005; MacKenzie, 2005). Occupancy models could obviously help to differentiate the true absence from the undetected presence of the species if the design could be implemented at the population scale. Karanth et al. (2011) successfully used such models with a tiger population because they could first stratify the sampling regarding habitat composition. On one hand, that very important stratification step might be difficult to perform with colonizing wolves that can live nearly in any habitat. Such a design would require a very costly and systematic sampling of cells with almost no chance to get any wolf signs there. On the other hand, applying such models on data derived only from the reproductive unit scale (e.g. Marucco and McIntire 2010) to build inference at the population scale rely on strong biological assumptions. The interaction between social driving forces and habitat use by a wolf within a pack versus a vagrant one likely shapes occupancy, so one can reasonably wonder whether inferring patterns from one scale to the other is robust enough.

Computing detection probabilities over space may strongly help in further refining the sampling protocol, but the reliability of model predictions may drastically decline with wolves colonising other habitats (incl. lowland with poor prey communities). High occupancy rates may reflect where the wolves are and might be, but low rates will not relate to unfavourable wolf areas. Also due to sparse data (number of recapture occasions per cell and per year), one might be bound to assume constant time-independent effects of factors, a very unlikely assumption given the colonizing context of a socially-regulated species.

However the decision making process needs to manage short term social and economic inter-

actions in the framework of such an expanding population. Therefore, it matters a lot organizing the data collection to obtain yearly updatable "coarse-grained" but robust proxies of the population dynamic. The sequential approach using first a spatially controlled network of field observers enables one to allocate the monitoring effort to pack detection before moving to the "pack scale" sampling design once the decision rule (presence detected over two consecutive winters) is achieved. Such a sequential survey provides empirical data to decision makers looking for updated information "quickly", based on rough proxies of population trends (e.g. minimum numbers detected from snowtracking). The main issue concerns detection probability, which is usually assumed to be constant (Pollock et al., 2002). To circumvent the difficulty of controlling the sampling effort (*sensu* Kindberg et al. 2009) over tens of thousands of square kilometres (current range of the wolf population), we chose first (at the "population" scale) to allocate the effort to the documentation of new areas of presence with an increasing number of trained experts and a control of their spatial distribution. Then the control of the sampling effort is investigated by highly-specialized people at the "reproductive unit" scale, where sets of transects or points are investigated according to an explicit and quantified sampling process. Another solution to get rid of unequal detectability, particularly with genetic longitudinal data sets, is to analyse them with MR models, and control a posteriori for the reliability of the demographic proxies derived from snowtracking (Marboutin and Duchamp, 2005). Due to possible stochastic events a given year (for example a winter season with poor snow cover resulting in a decrease in sign detectability), one would better be cautious in drawing inferences about population trends if they are based only on results from short series of data.

It is well known that low detection probabilities combined with small sample sizes may translate to low power to detect significantly changes e.g. in time-related abundance. A kind of stratification, whether model-based (Guisan et al., 2006) or data-based (as in our two-scale designs), in field data collection may help lowering the influence of imperfect detection, and improving

the ability to detect changes in population trend.

As the distribution range of the French wolf population may expand to the central and western part of France, we will probably face the issue of monitoring wolves in snow free areas where snow tracking is likely unfeasible. We may have to move to a simpler design, e.g. estimating only pack number and population distribution, rather than relative abundance, possibly based only on presence-absence data (Andren et al., 2002) as a rough surrogate to more classical demographic indices. In most of the cases, the detection of a new occurrence comes first from visual contacts or depredations, that both do not depend on snow. Therefore, the absence of snow may not strongly affect the detection of wolf presence. Surveying packs for presence-absence must include all types of reliable wolf signs recorded extensively, assuming absence when no data are recorded, provided some experts are surveying the site. The intensive survey (step 2) with transects can even be done but may provide very low detection probabilities in winter. Wolf howling sampling is then an interesting surrogate to intensively monitor presence at the reproductive unit scale (Ausband et al., 2010).

The monitoring design of such a controversial species has to provide robust data since they are often further used for management purposes (Roux et al., 2006). The dual frame survey we implemented seems relevant since it provides robust estimates of the population growth rate (Marescot et al., 2011), and therefore enables one to assess yearly the population status and to feed the decision-making process related to the management of wolf-related issues.

Lessons learned

- For conservation biology purposes, the scale dependent monitoring appears relevant to achieve both scientific and management purposes. The data-based stratification of field data collection into different scales provides the opportunity to build an explicit sampling strategy that is suitable for the allocation of sampling effort in the field.
- The dual frame also provides a cost ef-

fective way to address various issues. For instance, snow tracking allows for a better detection of scats, which in turn are used for non invasive molecular tracking, and finally for diet analysis.

- However, no one single probabilistic approach can fulfil all the requirements for proximate management purposes. We emphasize the need for a monitoring design that enable to document as quick as possible a new wolf occurrence for such management purposes, even though it does not always turn into a regular wolf territory later on. Given the context of a recovering population, the spatially-controlled network of experts is a key factor to address expectations of decision makers in charge of solving wolf-human activities conflicts.

- Observational data without any kind of methodological control is likely to result in biased estimates of population trends. A single coordinating structure (be it a governmental agency, a university laboratory, etc.) is the easiest way of standardizing the data collection, controlling for field effort and addressing biological interpretations. The contribution of various people with different backgrounds (state employees, hunters, NGOs, shepherds, etc.) within the network strongly contributes to a wider coverage of the survey effort both in space and time, provided a fairly regular distribution of people and a common technical filter for data validation. This also contributes to make the scientific results shared by all stakeholders (e.g. improves the social acceptance of results).

- In order to make the results meaningful to managers, yearly (at least) updates of the population status are sharply needed, at least during a colonizing phase. The sign surveys can be used to derive some yearly updatable proxies of local sub-units (i.e. packs). However, a calibration process must be carried out first (e.g. with non invasive genetics) to check for the reliability and sensitivity of those proxies. Whatever the case of large scale population-based or small scale pack-based survey, long term

monitoring is needed, first to discard over interpretations of an observed minor short term change in the population, and second to fulfill the requirements of mark-recapture models when estimating rate of increases accounting for detection heterogeneity (Cubaynes et al., 2010).

- Field biologists should also specify the level of uncertainty within their results, so that decision-makers take this into account. Sometimes, uncertainty is more explicit, or simply lower, in coarse-grained (but reliable) monitoring systems, for example when estimating only pack occurrence rather than occupancy or abundance. Accounting for all kinds of signs of presence, provided that a standard validation process is implemented, may balance the risks of under- versus overestimating the population status.

- Finally, we state that any wolf monitoring program may include a communication strategy. Within each district concerned by the wolf presence, we held annual meetings dedicated to local authorities and field experts, and a bulletin is published twice a year online (http://www.oncfs.gouv.fr/Le-Bulletin-du-reseau-loup-download130). Technical transparency making the same data available to every stakeholder is a key factor in elaborating a well-accepted decision making process.

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